JOURNAL OF AGRICULTURAL RESEARCH

VOL. 73 WASHINGTON, D. C., JULY 1, 1946 NO. 1

ESTIMATION AND PHYSIOLOGICAL ROLE OF SOLANINE IN THE POTATO [1]

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INTRODUCTION

It appears that the glycoalkaloid solanine occurs in several species of *Solanum* and in certain other genera of the Solanaceae, notably in *Lycopersicon*. In Europe a number of determinations have been made of the solanine content of cultivated varieties of the potato (*Solanum tuberosum* L.), but in the United States relatively little consideration has been given to the content of this alkaloid; this in spite of the available information on the dangerously high concentration of solanine in tuber "sprouts" and young shoots.

In general, there seemed to be a need for facts relating to the "physiology" of solanine occurrence, metabolism, and distribution in the various organs of the plant during development and maturity. Likewise, it may be suggested, further information is eventually desirable on problems affecting (1) the wholesomeness of the tubers (and of potato wastes in animal economy), (2) breeding programs, and (3) disease-resistance possibilities.

Recent studies on the chemistry of solanine have centered mainly on its molecular structure. Upon acid hydrolysis, solanine had been found to yield the aglycone solanidine and 1 molecule each of glucose, galactose, and rhamnose, which are attached to the aglycone in the order just enumerated (*11*, *12*, *17*, *26*)[2].

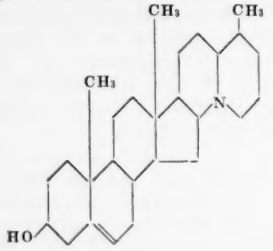
Soltys and Wallenfels (*22*) demonstrated by appropriate methods the presence of a sterol nucleus in solanidine which they suggested

[1] Received for publication October 19, 1944. This investigation, which forms part of a study of the physiology and disease resistance of the potato, was supported in part by the Wisconsin Alumni Research Foundation. Some assistance was provided from the personnel of Federal Works Project Administration. While the laboratory work was done in the Department of Botany, the writers are particularly indebted to the Department of Plant Pathology for supplemental support, and especially to Dr. J. C. Walker and Dr. R. H. Larson for cordial cooperation throughout, this cooperation making it possible to maintain field plots, to undertake greenhouse experiments otherwise impracticable, and to visit potato growers in the State for requisite material or for desirable observations. Thanks are also extended to Dr. V. W. Meloche for assistance on many chemical phases of the work, to Dr. G. H. Rieman for "seed material" of certain potato varieties for planting and analyses, and to Eugene Herrling for critical work on the graphs.

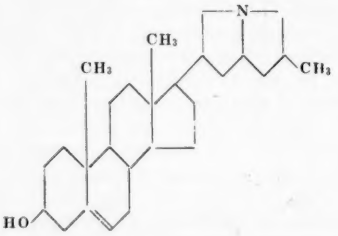
[2] Italic numbers in parentheses refer to Literature Cited, p. 31.

was similar to that of cholesterol. The more recent studies of Rochelmeyer (*20*) on the homocyclic portion of potato solanidine (called "solatubine" by this author) confirmed this view, and demonstrated that all of the known derivatives of solanidine show the same relationship with respect to the position of the double bond and the configuration as in the cholestane series.

The structure of the nitrogen-containing portion of the molecule, which is still in doubt, was assumed by Soltys and Wallenfels to form a lupinane structure (A, in the formula presented) because of its resistance to degradation, but Clemo et al. (*7*) favor another structure (indicated in B of the formula) in which a methyl pyrrolizidine [3] is attached to the sterol nucleus at only one point, that is, at C^{17}. The structures suggested for solanidine are the following:



A



B

In addition to solanine, the two derivatives solanidine and solanthrene have been reported in tissues (*8*, *13*). It is not clear that solanthrene occurs as such in tissues; in fact, this substance may be produced as the result of a side reaction by the dehydration of solanidine during the acid hydrolysis of solanine

The bitter taste and hemolytic action of the glycoalkaloid solanine, with its consequent effect on the wholesomeness and quality of the potato, have aroused considerable interest among European workers. The solanine content of the tubers of a large number of potato varieties commonly grown in Europe has been investigated, and toxic concentrations have at times been reported (*5*, *10*, *21*, *23*, *24*).

In the present paper a survey of the solanine content of a number of potato varieties grown in the United States is reported, and some of the environmental factors affecting the production of solanine in the various organs of the potato plant are described. While existing methods of analysis of tissues for solanine were utilized as far as practicable, such methods were found inadequate in some respects, and considerable time has, therefore, been devoted to the development and improvement of procedures for quantitative extraction and assay. Also, it was necessary to have pure solanine or its derivatives for colorimetric assay. Solubility studies of solanine were essential in working over the gravimetric procedure of Bömer and Mattis (*5*).

[3] This compound has been synthesized recently by Clemo and Melrose (*6*). A third structure for the nitrogen-containing portion of the molecule is proposed by Prelog and Szpilfogel (*19*).

PREPARATION AND PROPERTIES OF THE POTATO ALKALOIDS

In the course of this work, pure solanine was prepared from tissues by a number of methods. Leaves and sprouts, used chiefly because of their high alkaloid content, were extracted in both the fresh and the dry state. Fresh tissues were ground, covered with 2 percent acetic acid, and allowed to steep for 2 to 4 days. The pulp was then pressed out, the extract was made strongly ammoniacal and heated to about 70°C. The precipitate which formed was filtered and dried.

A similar, crude product was secured by using dry, powdered leaf tissues. In this case, the powder was extracted twice by boiling it for about 1 minute with 1 percent acetic acid. The mixture was filtered and then treated with ammonia as indicated above. The precipitate in either case was extracted by refluxing with alcohol and filtering the hot alcoholic extract Upon cooling, the solanine crystallized out of the filtrate in small loose needles or formed a gel. If considerable hydrolysis of the glycoside had occurred during extraction, the precipitate frequently consisted of a mixture of solanine and solanidine from which the solanidine was readily removed with ether.

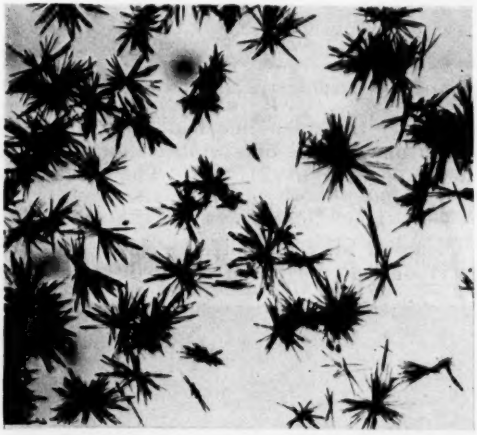


FIGURE 1.—Solanine crystallized from alcohol. × 32.

After the first crop of crystals had been filtered off, the resulting filtrate containing solanine, solanidine, and solanthrene was concentrated and treated with ammonia in order to secure maximum yields of the nitrogenous bases. The heavy precipitate which formed was filtered, dried, and refluxed with ether. The residue, consisting mainly of solanine, was purified by repeated recrystallization from alcohol. The final product (fig. 1) melted with decomposition at 285° to 287° C.

The ether extract containing the solanidine and solanthrene was evaporated to dryness. The residue, consisting of very large, coarse needles, was purified by repeated recrystallization from alcohol or

acetone. The latter solvent appeared to be more effective in separating these two bases. In most cases the solanidine precipitated out as long needles (fig. 2), leaving the bulk of the solanthrene in the super-

FIGURE 2.—Solanidine crystallized from alcohol. × 25.

natant liquid. After the solanidine had been purified by repeated recrystallization from alcohol or acetone, the final product melted with slight decomposition at 217° C. The specific rotation $[\alpha]_D^{23}$, was −35.8° ($[\alpha]_D^t = \frac{100a}{lc}$, $a = -0.23$, $l = 2$, $c = 0.3162$ gm. per 100 ml. of alcohol). Clemo et al. (7) reported a value of −28.5° for $[\alpha]_D^{21}$. The monoacetyl derivative (fig. 3) formed by heating solanidine

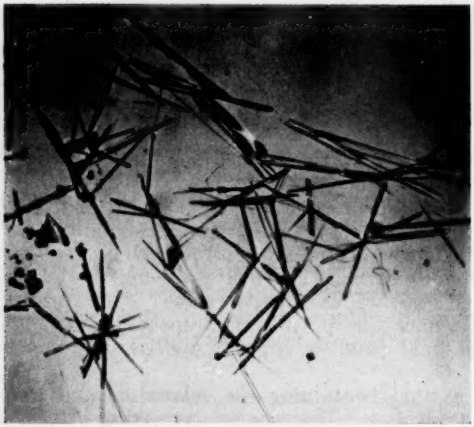


FIGURE 3.—Acetyl solanidine crystallized from alcohol. × 50.

with acetic anhydride for 30 minutes and purifying by recrystallization from alcohol melted at 203°–205° C. (Clemo et al. (7) give 203° C.).

The solanthrene could be recovered by concentrating the original mother liquor from which the solanidine had been derived and purifying the product which precipitated by repeated recrystallizations from acetone or alcohol. In acetone, solanthrene crystallized out in very large, coarse needles (fig. 4) which melted at 167°–168.5° C.

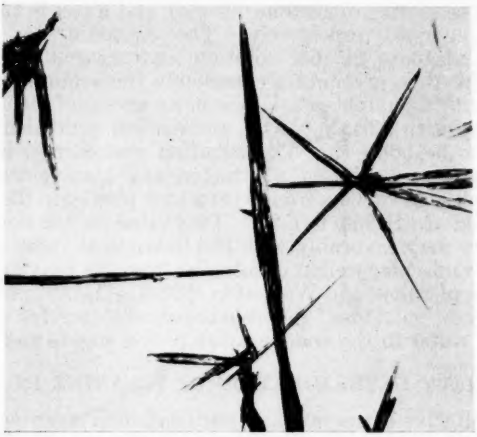


FIGURE 4.—Solanthrene crystallized from acetone. × 20.

This procedure, although yielding satisfactory products, was suitable for working over only relatively small quantities of material under the conditions prevailing in these experiments. The extraction step was subsequently modified by extracting the dry powdered leaves or sprouts directly with hot alcohol in a Tutin type extractor (*2*). The bases were precipitated from the extract by the addition of ammonia, followed by filtering and then extracting the precipitate by refluxing with alcohol. The alkaloids present in the alcohol were separated and purified in the manner indicated above.

Solanidine and solanthrene were also prepared by hydrolyzing solanine in 4 percent hydrochloric acid, and separating and purifying the hydrochlorides of the two bases by appropriate means.

An aqueous, 0.0379 molar solution of solanine, prepared as indicated above, and containing a very slight excess of hydrochloric acid, produced a blue fluorescence excited by a considerable range of the ultraviolet region. The maximum intensity was at about 300 to 350 mμ, and it disappeared at about 227 mμ. An alcoholic solution of solanidine, 0.0121 molar, behaved similarly, displaying the same maxima as an aqueous solution of solanine, so that the aglycone alone was responsible for the fluorescence.

With the Liebermann-Burchard reagent, solanine produced a cherry-red to violet-red color, changing slowly to violet and finally to olive green. Solanidine gave a fleeting red color followed by an olive

green. With the same reagent solanthrene quickly developed a red color which remained for ½ to 1 minute, and was replaced by a permanent bright-green color. Colors were also produced with the 3 bases and with the reagents of Fröhde, Brandt, and Marquis. The last served as the basis of the colorimetric determination of solanine in potato tissues, and will be described in detail later.

With cholesterol, solanine formed a characteristic gellike cholesteride in alcoholic solution. Solanidine formed an insoluble digitonide with digitonin. In the latter, precipitation occurred within a few minutes in a medium consisting of acetone, alcohol, and water in the proportion 20:71:9 by volume, respectively. The digitonin was added as a 0.5 percent absolute alcohol solution, in excess of the theoretical amount required to precipitate completely the solanidine.

By dissolving a sample of solanine in an excess of 0.01 N HCl, and back-titrating with 0.01 N NaOH, the neutral equivalent of solanine was found to be 906 ± 12. The titration was carried out potentiometrically with a Beckman pH meter and glass electrode. With samples of solanine varying from 0.0802 to 0.1506 gm., the equivalence point occurred at pH 5.80 to 6.23. This value for the neutral equivalent compares very favorably with the theoretical value of 885 calculated for the molecular weight of solanine from the provisional formula for solanidine of Soltys and Wallenfels (*22*) ($C_{27}H_{43}ON$, plus 1 molecule each of glucose, galactose, and rhamnose, allowing for the loss of 3 molecules of water in the condensation of the sugars and solanidine).

SOLUBILITY DETERMINATION OF SOLANINE IN WATER

The quantitative extraction and purification of solanine involves its precipitation from an alkaline medium. Bömer and Mattis (*5*), working with solutions of undetermined pH, introduced a solubility correction of 2.75 mg. of solanine per 100 ml. of alkaline filtrate. Subsequent investigators, accepting this value, applied the same correction in reporting their analytical results. Since the correction may frequently exceed the total solanine actually recovered, an exact knowledge of the solubility of solanine at higher pH levels is of considerable importance in the quantitative estimation of the alkaloid.

The solubility of solanine varies with the pH of the solution. Using the violet-red color produced with solanine by sulfuric acid and formaldehyde, the writers made numerous attempts but failed to obtain a positive test for solanine in alkaline filtrates of pH higher than 9.3, although the method will readily detect 0.5 mg. of the glycoside in 100 ml. of solution. Furthermore, solubility product considerations indicate that at pH 9.5, the solubility of solanine should be of the order of 0.1 mg. per 100 ml.

The solubility product constant of solanine, K_{sp}=solanine.$H^+ \times OH^-$, was calculated from the neutralization curve (fig. 5) secured by titrating solanine.HCl against standard sodium hydroxide. The concentration of solanine.H^+ at any point on the curve was calculated from the volume of standard alkali employed, and the hydroxyl-ion concentration was calculated from pH values at the same point. The pH was secured by means of a glass electrode. The average value for the solubility product was found to be 4.27×10^{-11}. Kolthoff (*14*) had secured a value of 6.4×10^{-12} for the solubility product constant of

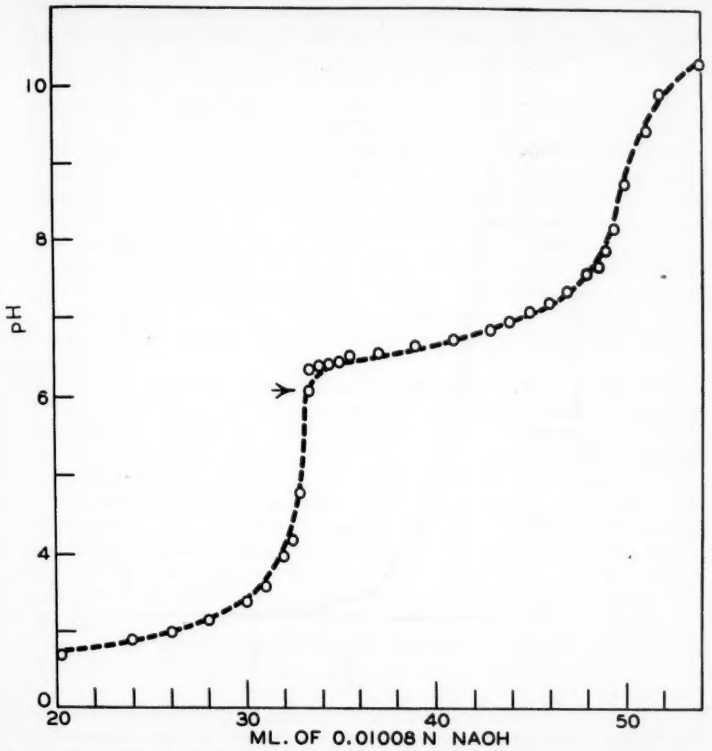


FIGURE 5.—Neutralization curve of solanine.HCl; 0.1506 gm. of solanine dissolved in an excess of 0.0100 N HCl. The free base begins to precipitate at about pH 6.2, and precipitation is complete at about pH 9.3.

solanine. Using the figure 4.27×10^{-11}, the concentration of solanine.H^+ was calculated for various points from pH 6.4 to 9.5, and plotted in figure 6. Curve *b* in this figure was secured by the colorimetric method, employing the sulfuric acid-formaldehyde reagent. For this purpose, a series of saturated solutions of pure solanine were prepared by dissolving a small quantity of the alkaloid in 150 ml. of water containing from 1 to 4 ml. of glacial acetic acid, and carefully adjusting with ammonium hydroxide to form a series ranging in pH values from 6.4 to 9.5. The precipitate which formed in each case was allowed to stand in well-stoppered bottles for 16 hours to 2 days before being filtered. The solanine concentration in the filtrate was determined colorimetrically, and the pH was observed with the Beckman meter. The two curves obtained by independent methods agree fairly well, particularly at the higher pH levels where the solubility of the alkaloid has dropped practically to zero, and where the solubility product principle might be expected to apply most rigorously.

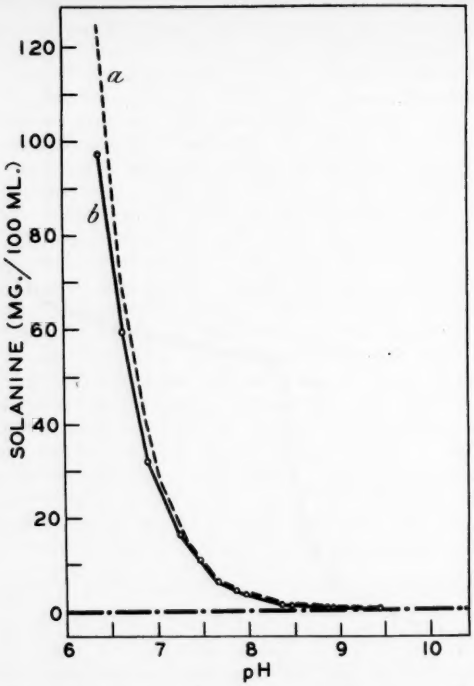


FIGURE 6.—Curves showing solubility of solanine under equilibrium conditions at various hydrogen-ion concentrations: *a*, Values of the concentration of solanine.H^+, calculated from $K_{sp}=4.27\times10^{-11}$; *b*, solanine concentrations at various pH levels, determined colorimetrically.

Kolthoff (*14*) has reported a solubility of 25 mg. per liter of the free base (solanine.HOH) in water. This was checked by adding various amounts of 0.0100 N sodium hydroxide to solutions of solanine hydrochloride. This salt was prepared by shaking an excess of pure solanine in a volume of 0.0100 N hydrochloric acid until equilibrium was established. The undissolved alkaloid was filtered off and 15-ml. aliquots of the filtrate were diluted to 100 ml. with carbon dioxide-free distilled water. Small, carefully measured volumes of standard sodium hydroxide were added, with shaking, to each solution of the series in progressively increasing amounts. A precipitate formed in all cases where alkali was added.

Solanine hydrochloride is assumed to dissociate as follows:

$$\text{Solanine.HCl} \rightleftharpoons \text{Solanine.H}^+ + \text{Cl}^-$$

In the presence of sufficient hydroxyl ion, solanine.H^+ forms the free base

$$\text{Solanine.H}^+ + \text{OH}^- \rightleftharpoons \text{Solanine.HOH}$$

From a knowledge of the equivalence of solanine, and the quantity of standard alkali needed to induce incipient precipitation, the solubility of the free base in water may be calculated.

Because of the extremely low solubility of the free base, only a rough estimate of the upper limit of solubility can be made from this experiment. As little as one drop of 0.0100 N sodium hydroxide added to 100 ml. of the hydrochloride solution was sufficient to cause precipitation, so that the solubility of the free base must be less than 0.45 mg. per 100 ml. For all practical purposes, the free base may be considered insoluble in water.

The same conclusion can be arrived at independently from a study of figure 6. Since the colorimetric method will detect any solanine in the form of the free base as readily as solanine .H^+, the close agreement between the two curves indicates that solanine .HOH may be disregarded.

More direct evidence is obtained by actual estimation of solanine losses upon precipitating solanine out of alkaline solution. Samples of pure solanine (melting point 285–286° C., dec.) weighing from 32.1 to 44.3 mg. were dried at 85° C. to constant weight, and dissolved in 0.4 percent acetic acid. The solution was then adjusted to pH 10.0 to 10.5 with ammonia. After digesting on the water bath for 30 minutes, the precipitate which formed was filtered, washed with ammonia water of approximately pH 11.0, and dried to constant weight. In 6 such determinations involving ammoniacal filtrate volumes of 76 ml. to 335 ml., with final pH values that ranged from 9.55 to 10.34, total losses of exactly 0.9 mg. occurred in each of 2 samples; in the remaining 4 the original solanine which was added was quantitatively recovered. The 0.9 mg. losses reported in the above 2 cases could not be due to solubility since the ratio of the volumes of the ammoniacal filtrates in the two cases was more than 4 to 1.

Similar tests carried out as nearly as possible under the conditions described by Bömer and Mattis (*5*) always resulted in a loss of solanine. The quantity lost was variable, but amounted to as much as 1.8 mg. in a total volume of 126 ml. of ammoniacal filtrate. Although these investigators did not determine the pH of their solutions, it is probable from the present studies that they precipitated their product within the range pH 8.9 to 9.2. The writers have noted that solanine is not completely precipitated until a pH of 9.3 is exceeded, so that a part of the solubility Bömer and Mattis reported can be attributed to carrying out their precipitations at too low a pH. Also, apparent losses of solanine might occur after precipitation if any impurities were present in the original preparation Furthermore, since they heated their precipitates at 105° C., the possibility of partial decomposition of the glycoside during prolonged heating should not be excluded.

Solanine forms a gelatinous precipitate which is extremely difficult to handle quantitatively. It is suggested that the slight losses of alkaloid which sometimes occur in quantitative estimation of the product are more properly regarded as mechanical losses than as a measure of the solubility of solanine in alkaline medium.

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QUANTITATIVE DETERMINATION OF POTATO ALKALOIDS

TUBER ASSAY

Potato tubers were assayed by a modified Bömer and Mattis procedure, incorporating certain changes. Four or more tubers, depending on size, were finely ground in a Nixtamal mill. The resulting pulp was assayed in duplicate by thoroughly mixing it for about 5 minutes and then quickly pouring off 2 portions of approximately 250 gm. All weighings were made to within 1 gm.

According to the procedure followed, a volume of 0.25 percent acetic acid equal to the weight in grams of the pulp sample is added, and the mixture is allowed to stand at room temperature with occasional stirring for ½ to 1 hour, after which it is squeezed out through 3 thicknesses of clean cheesecloth in a hydraulic press at 5,000 pounds per square inch. The pad of ground tissue is extracted three more times in the same manner. Approximately 250 ml. of 0.25 percent acetic acid is added, and 1 hour is allowed for each extraction, after which the tissue is squeezed in the press. The combined extracts are made slightly ammoniacal as soon as they are prepared, to prevent enzyme activity. Finally 10 gm. of diatomaceous earth are added and the mixture is well stirred.

The extract is then placed in the oven at about 85° C. to evaporate. When only a small amount of the liquid remains, the sides of the container are washed down with a small quantity of 0.25 percent acetic acid, a slight excess of ammonia is added, the mixture is well stirred, and the remaining liquid is evaporated off. The dry residue is scraped out of the container, ground in a mortar with a pestle, and extracted with 95 percent alcohol in a Soxhlet extractor for approximately 18 hours. The residue is then reground and extracted with 95 percent alcohol for another period of 18 hours. A third alcohol extraction of the residue yielded no more solanine, so that two 18-hour periods were adopted. Valentin (*23*) extracted for 24 hours, during which time the material was reground twice.

The combined alcoholic extracts are evaporated to dryness and the residue is taken up quantitatively with several 25-ml. portions of 0.25 percent acetic acid. These are filtered, and the combined filtrate totaling from 100 to 150 ml. is adjusted to a pH of 10.0 to 10.4 with ammonia. The solanine is precipitated from this alkaline solution by digesting over a boiling water bath for 20 to 30 minutes. The alkaloid is collected on a filter and purified by redissolving it with approximately 100 ml. of 0.25 percent acetic acid, adjusting to pH 10.0 to 10.4 as before with ammonia, and precipitating on the water bath. Earlier investigators made no effort to control the pH of the medium during precipitation of solanine. After three, or at the most four, such purifications a perfectly white precipitate of solanine is obtained from a clear solution. This is collected on a tared filter paper, washed with three 15-ml. portions of an approximately 2 percent solution of ammonia, and weighed after being dried to constant weight at 85° C. All weighing was done in closed weighing bottles.

The product prepared in the manner described above may contain small quantities of solanidine and solanthrene, if any are present in the tissues, but it is free of ash. Numerous tests indicated a decom-

position point of approximately 240° C. (m. p. of pure solanine, 285° C., dec.). As stated above, the procedure includes some departures from the Bömer and Mattis (*5*) method. The most significant of these was precipitating the alkaloid in the purification steps from solutions of controlled pH and eliminating the solubility correction. In order to effect complete extraction of the dried residue, it was found necessary to extend the period of Soxhlet extraction from two 5-hour periods to two 18-hour periods. Also the final alcohol treatment of the precipitate was replaced by an additional precipitation from ammoniacal solution.

The standard error of the method, $\frac{\sigma}{\sqrt{N}}$, was ± 0.120 mg. of solanine per 100 gm. of fresh tissue.

An effort was made to shorten the assay of tubers by direct drying of a weighed portion of the fresh pulp at 90° to 95° C., pulverizing and extracting the residue with alcohol, and proceeding from this point as in the procedure described above. Parallel determinations made by the two methods on the same tuber samples showed that the shorter method in most cases gave much lower yields of alkaloid, and was, therefore, abandoned.

ASSAY OF OTHER ORGANS

Tissues other than tubers were dried at 85°–90° C. after collection. After 48 hours, the dried tissues were ground in a Nixtamal mill or in a mortar with a pestle. The powdered material was stored in stoppered bottles until needed, when from 0.5 to 5 gm. were dried at 85° C. in weighing bottles and weighed.

The sample was extracted three times by boiling it for ½ to 1 minute with 50 ml. of 1 percent acetic acid. Further extraction of the residue yielded no additional solanine. After each extraction the mixture was filtered under moderate suction, and the residue was washed with 10 ml. of 1 percent acetic acid. The pH of the combined extracts was adjusted with ammonia to 10.0 to 10.4, and the solution was digested on the water bath for 30 minutes. The flocculent precipitate was filtered, using No. 42 Whatman paper and light suction. The paper and residue were extracted three times by being boiled in approximately 25-ml. portions of absolute alcohol. The combined alcoholic extracts were evaporated to dryness over the water bath, and the residue was taken up with about 50 ml. of 0.5 percent acetic or sulfuric acid and filtered. The filter paper was washed three times with approximately 10-ml. portions of 0.5 percent acetic acid or sulfuric acid and the combined filtrates and wash solutions were made to volume in a 100-ml. volumetric flask. Stem tissues which normally ran very low in solanine were usually made up to a final volume of 50 ml. The solutions in all cases were colorless.

The solanine in these solutions was estimated colorimetrically by a method employed semiquantitatively by Alberti (*1*) and adapted to the quantitative estimation of solanine in potato tuber extracts by Pfankuch (*18*). Six milliliters of concentrated sulfuric acid were added dropwise from a burette to a 3-ml. aliquot of the acid extract while it was being rotated vigorously in an ice bath. The yellow solution which formed was allowed to stand for 1 minute, and 3 ml.

of 1 percent formaldehyde were then added dropwise with constant rotating as before. With the addition of formaldehyde, the yellow color changed to orange, then to red, and finally to a violet red. The optical density, log I_0/I, of this solution, which becomes stable after about an hour and a half, was estimated in an Evelyn photoelectric colorimeter using filter 540. The 1 percent formaldehyde was carefully prepared from commercial formalin by dilution, the formalde-

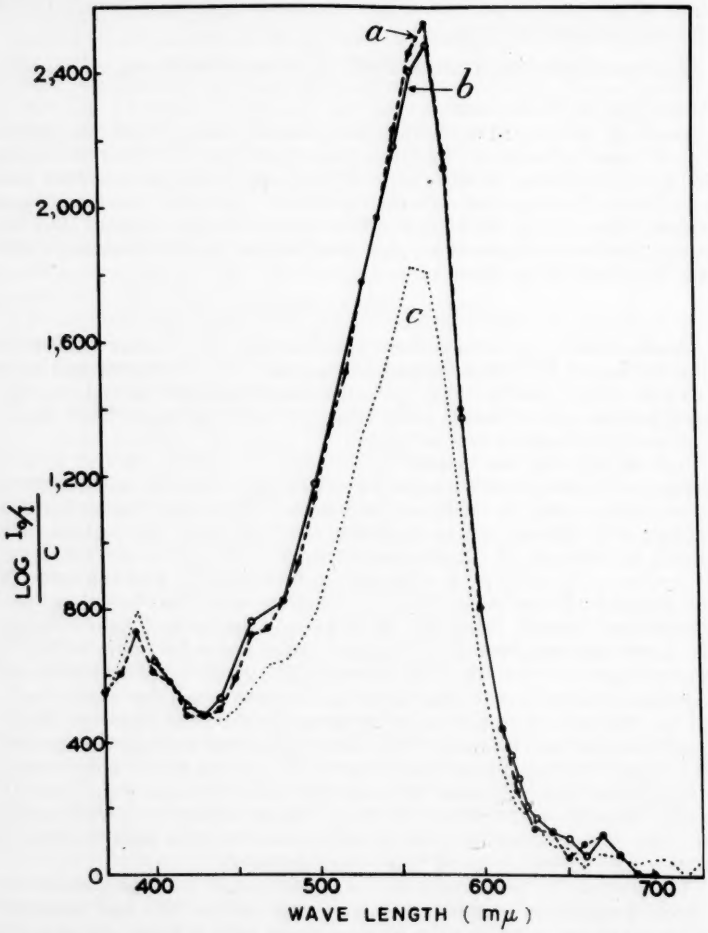

FIGURE 7.—Curves showing molar absorption spectra of the violet red color formed by solanine, solanidine, and solanthrene with sulfuric acid and formaldehyde solution: *a*, Solanine, $C=2.17\times10^{-4}$ moles per liter; *b*, solanidine, $C=2.51\times10^{-4}$ moles per liter; *c*, solanthrene, $C=3.11\times10^{-4}$ moles per liter.

hyde content of the latter having first been determined by the method of the Association of Official Agricultural Chemists (*3*).

The molar absorption spectra of the violet red color formed by sulfuric acid and formaldehyde with solanine, solanidine, and solanthrene are indicated in figure 7. The absorption spectra were determined with a Cenco-Sheard spectrophotelometer using wave lengths at 10-mμ intervals in the visible range. The absorption cell used with this instrument had a depth of 1 cm. so that the quantity $\frac{\log I_0/I}{C}$ is the molar extinction coefficient. Solanine and solanthrene were dissolved directly, the latter with considerable difficulty, in 0.5 percent acetic acid, but solanidine was first dissolved in 0.5 ml. of glacial acetic acid, and diluted to 100 ml. with distilled water. The color was developed in these solutions in the usual manner. The molar extinction coefficients of solanine and solanidine are seen to coincide very closely at all wave lengths, indicating that the production of the violet red color is independent of the sugars on the solanidine nucleus. Solanthrene, on the other hand, has a much lower absorption in the green, yellow, and orange region of the spectrum. The general form of the absorption curve, however, is the same in all three compounds, exhibiting a maximum at 560 to 570 mμ. A filter for the Evelyn colorimeter with maximum transmission in this range was not available, and, therefore, the 540 filter was used as indicated above, in which approximately 95 percent of the light transmitted is between 515 and 570 mμ.

The calibration curve shown in figure 8 was prepared with filter 540, by plotting the optical densities of properly prepared known solanine solutions against the solanine concentration.[4] It is clear that the values obtained conform fairly well to Beer's law, particularly those of log I_0/I from 0.05 to 0.8. From the curve, the value of the combined constant kl (slope), in the equation

$$\text{Log } I_0/I = klc$$

is 4.7 so that the concentration of solanine (c, in mg. per ml.) in unknown solutions may be secured from the curve, or it may be calculated by dividing the optical density by 4.7. The most accurate results are obtained at concentrations of 0.01 to 0.09 mg. of solanine per milliliter, and readings were generally taken within this range, the solutions being diluted if necessary.

The method was tested by mixing 5.5 mg. of the solanine with approximately 3-gm. samples of White Rural leaf powder. The average recovery was 5.4 mg. For comparison, leaf samples were also assayed gravimetrically, using the same procedure as above, except that an aliquot of the final acid solution was assayed colorimetrically, while the alkaloid was precipitated quantitatively out of the remainder of the solution and weighed after being dried to constant weight. A comparison of results secured by the two methods is shown in table 1.

[4] In keeping with standard procedure, the value I_0 was secured by setting the galvanometer scale at 100 when a blank composed of 3 ml. of 0.5 percent sulfuric acid (or 0.5 percent acetic acid), 6 ml. of concentrated sulfuric acid, and 3 ml. of 1 percent formaldehyde was in position.

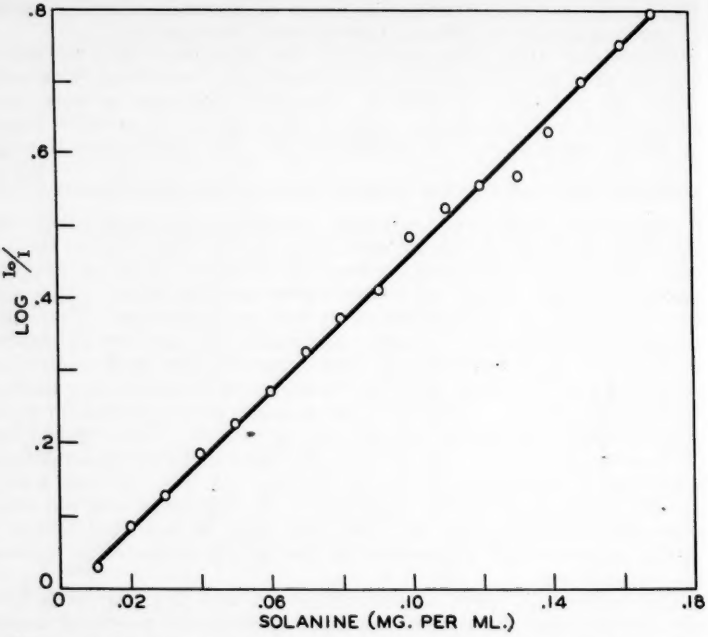

FIGURE 8.—Calibration curve illustrating close agreement with Beer's law of the colored solution formed by solanine with concentrated sulfuric acid and formaldehyde solution.

TABLE 1.—*Solanine concentration in powdered leaf tissues of White Rural potato as determined by the colorimetric and gravimetric methods*

Dry weight of leaf sample (grams)	Total solanine by—	
	Colorimetric method	Gravimetric method
	Milligrams	*Milligrams*
2.587	29.4	29.3
2.544	24.6	24.8
2.556	22.0	22.5
1.311	14.2	14.5
1.320	14.2	14.7
1.297	15.1	14.1
1.311	15.0	14.5
1.297	13.9	14.1
1.300	14.5	14.7

DISTRIBUTION OF SOLANINE IN POTATO TUBERS

Various factors must be taken into consideration in a comparative study of the solanine content of tubers. In order to standardize tuber-sampling procedure a study was made of the relation between solanine content and tuber size. Data for two varieties are shown in table 2. In general, an inverse relationship was found between tuber

size and solanine concentration, although, as might be expected, the total solanine per tuber shows a tendency to rise with increase in tuber size, at least up to a total weight of about 135 gm. For comparative studies, a tuber weight of 120±10 gm. was arbitrarily adopted, and, unless otherwise stated, results are always reported on this basis. From the data reported it is obvious that analyses of solanine content of varieties is of little comparative value unless tuber size is taken into account.

The storage of tubers at 4°–5° C. for a long period after harvest does not appreciably affect the solanine content of the varieties tested, but analyses should be made within 6 or 7 months because a slight rise is observed beginning in April or May and it is obvious that, when the solanine content is determined on a fresh-weight basis, any water loss from the tuber leads to an apparent increase in solanine.

TABLE 2.—*Relation between tuber size and solanine content*

Variety	Average tuber weight	Average solanine (milligrams per 100 gm. of fresh tissue)	Total solanine per tuber
	Grams		*Milligrams*
White Rural	31.3	18.0	5.63
	62.6	17.3	10.8
	93.2	16.9	15.7
	135	14.8	20.0
	150	12.6	18.9
	178	13.0	23.1
	210	8.9	18.7
	270	7.3	19.7
Irish Cobbler	32	7.8	2.49
	265	3.7	9.80

The distribution of solanine in the tissues of White Rural and Irish Cobbler is shown in table 3. The solanine concentration is arranged in a descending gradient from the outside inward, with by far the highest accumulation of the glycoside in the peripheral (periderm and outer parenchyma) zone. None was found in the pith, by the procedure outlined above for tubers, on a 250-gm. sample of pith tissue. Within the peripheral zone, the highest solanine concentration occurs in the eyes, but a relatively high concentration is also found in the eye-free portions. The absolute magnitude as well as the ratio of solanine in eyes to eye-free tissue in the peripheral zone is of interest. In Irish Cobbler, this ratio is approximately 3, whereas in White Rural it is less than 2, indicating a more uniform distribution of the alkaloid throughout the thin peripheral zone in the latter variety.

The peelings assayed in this experiment correspond to the thickness of peel commonly cut away in preparing tubers for cooking. This process removes approximately 61 to 73 percent of the solanine present in the tuber, so that there is little danger of toxic effects in eating normal tubers (of the varieties tested) prepared in this manner. Griebel (*10*) reported that 29.7 to 35.7 percent of the original solanine was removed in peeling, while Meyer (*15*) gives a value of 44.1 percent. Bömer and Mattis (*5*) estimate, as a result of their work, that

41.2 to 80.1 percent of the solanine is discarded with the peelings. Obviously, in such analyses comparable samples are scarcely practicable, and a considerable variation may be attributable to this fact.

TABLE 3.—*Solanine distribution in potato tubers*

Tissues	Solanine (milligrams per 100 gm. of fresh weight)	Total solanine per tuber	Solanine removed in peeling
White Rural:		*Milligrams*	*Percent*
Periderm and cortex, including eyes	53.0		
Pith	[1] None		
Remaining tissues between pith and cortex	2.98		
White Rural: [2]			
Periderm with part of cortex, 1 to 2 mm. thick	48.6	9.97	72.7
Eyes, 6 to 12 mm. in diameter	81.9	3.37	
All remaining tissues	4.49	5.03	
Total		18.37	72.7
Irish Cobbler: [2]			
Periderm with part of cortex, 1 to 2 mm. thick	15.7	3.01	61.7
Eyes, 6 to 12 mm. in diameter	45.4	2.11	
All remaining tissues	1.64	2.19	
Total		7.31	61.7

[1] In 250 gm. of tissue.
[2] Average weight of White Rural tubers, 137 gm; of Irish Cobbler tubers, 159 gm.

RELATION OF POTATO VARIETY TO SOLANINE IN TUBERS

A survey of the solanine content of the tubers of 32 varieties of potato was made. In view of the variability in concentration observed in different soil types, it would have been desirable, for purposes of comparison, to analyze potatoes grown in the same locality. However, all 32 varieties were not available from a single area, and, therefore, most of the samples were secured from two Wisconsin localities, Rice Lake and Port Wing. *Solanum commersonii* was grown at Madison. The range of solanine concentration for cultivated varieties is seen to extend from 1.8 to 13 mg. per 100 gm. of fresh tissue (table 4). This compares with a range of 2 to 10 mg. reported by Bömer and Mattis (23 German varieties). It must be borne in mind, however, that these authors added a correction of 0.91 to 2.2 mg. per 100 gm. of fresh tuber to all of their results, depending on the total volume of alkaline filtrate from which the solanine had been precipitated during the purification procedure. Wintgen (*25*) also reported a normal range of 2 to 10 mg. per 100 gm. of fresh material (11 German varieties). Von Morgenstern (*16*) analyzed 39 varieties of potatoes and found that the solanine concentration extended over much wider limits, from 4.6 to 35 mg. per 100 gm. of fresh tissue (0.0046 to 0.035 percent).

There is no obvious correlation between time of maturing and solanine content. *Solanum commersonii*, however, is very late-maturing, flowers continuously, and remains active until killed by frost. The alkaloid extracted from this species gives the same color reactions (sulfuric acid-formaldehyde reagent and Liebermann-Burchard test) as potato solanine. Furthermore, the molar absorption spectrum of the colored solution formed with the former reagent

TABLE 4.—*Solanine content of tubers of different varieties of potato grown during the summer of 1938 at Rice Lake and Port Wing, Wis.*

Variety	Average solanine content (milligrams per 100 gm. of fresh tuber)	Time of maturation
Columbia Russet	1.8	Late.
Russet Rural	2.1	Do.
Sebago	2.1	Do.
McCormick	2.6	Do.
Jubel	3.2	Medium late.
Triumph	3.6	Early.
King	3.7	Midseason.
Brown Beauty	3.7	Do.
Earlaine	3.9	Do.
Green Mountain	4.0	Late.
Wee McGregor	4.2	Midseason.
Russet Burbank	4.7	Late.
Early Ohio	4.8	Early.
Mesaba	5.2	Do.
Hundred Day Cobbler	5.3	Do.
Arnica	5.4	Medium late.
Katahdin	5.5	Late.
Irish Cobbler	5.6	Early.
Ackersegen	5.8	Medium late.
Cobbler (greened)	6.4	Early.
Houma	6.4	Late.
Chippewa	6.6	Midseason.
Warba	6.9	Early.
Blue Salad	7.0	
Early Rose	7.2	Early.
Golden	8.8	Midseason.
Early Epicure	8.9	Early.
White-blossomed Cobbler	9.4	Do.
Pioneer Rural	10.0	Late.
Erstling	10.0	Early.
Hindenburg	11.6	Late.
Rural New Yorker (White Rural)	13.0	Do.
Solanum commersonii	[1] 508.0	Do.

[1] Small tubers only are produced.

is identical with that of potato solanine. After two recrystallizations from alcohol, the product had a melting point of 283° (dec.). The mixed melting point with a sample of pure potato solanine (m. p. 285° C., dec.) was 281° C. It would seem, therefore, that the alkaloid of *S. commersonii* may be identical with that of *S. tuberosum*. This problem is being further investigated. It is quite probable that *S. commersonii* matures so slowly that it is still unripe at the end of the season, and the high solanine concentration may be in small part due to this condition and to the small size of the tubers, although the species normally produces a very large amount of the alkaloid. A large proportion of the substance found in the tubers is probably supplied by transport from the tops.[5]

INFLUENCE OF LIGHT ON SYNTHESIS AND DISTRIBUTION OF SOLANINE

Fifteen White Rural tubers, each weighing 120±2 gm., were selected for this study. As a control 5 of these tubers were assayed for solanine immediately; the remaining 10 were planted in sand in 8-inch pots. Five of these were left in the light, and the remaining 5 were kept in a dark, well-ventilated chamber in the greenhouse.

[5] Recent work of Bell, Briggs, and associates (*4*) indicate that the alkaloid of *S. auriculatum* and *S. aviculare* is not identical with solanine.

After 35 days, the plants grown from these tubers were harvested and the old ("mother") tubers saved for analyses. A few stolons had formed by this time, but tuberization had not set in. Tops and roots were dried at 85° C., weighed, ground, and analyzed for solanine according to the method described for such tissues. The mother tubers were handled in the usual way recommended for tubers. The mother tubers had gained weight during this period, but results are reported on the basis of the original weight. The solanine content of the first or control group of five tubers which was assayed was 16.7 mg. per 100 gm. of fresh weight. It was assumed that this was also the initial solanine content of the potatoes which had been planted. Table 5 shows several points of interest. The solanine values of tops and roots are within the range expected. However, the change in alkaloidal content of the mother tubers between the beginning and end of the experiment is relatively slight. Apparently no appreciable translocation of the glycoside from the mother tubers could have occurred, and it seems reasonable to believe that the solanine isolated from tops and roots must have been produced in those organs. Furthermore, a large amount of the alkaloid was formed in the dark, indicating that in the presence of adequate supplies of raw materials the synthesis of the glycoside is qualitatively independent of light.

TABLE 5.—*Effect of light and darkness on solanine synthesis in tops and roots of White Rural potato*

Part of plant	Solanine (milligrams per 100 gm. of dry weight)	Solanine (milligrams per 100 gm. of fresh weight)	Total solanine in 5 hills	Dry weight 5 hills
	Light			
			Milligrams	*Grams*
Tops	1,290	131	267	20.5
Mother tubers		13.7	76.6	
Roots	863	57.0	112	13.0
Total [1]			379	33.5
	Darkness			
Tops	1,030	63.5	180	17.3
Mother tubers		16.5	93.7	
Roots	1,170	85.0	49.0	4.20
Total [1]			229	21.5

[1] Total does not include solanine in mother tubers, which is assumed to be, as found in the controls, 16.7 mg. per 100 gm. of fresh weight.

It is of interest to note that the solanine concentrations on a dry-weight basis are as follows: In combined tops and roots in darkness, 1.06 percent; in combined tops and roots in light, 1.13 percent; in tops alone in light, 1.30; in roots alone in light, 0.86; in tops alone in darkness, 1.03; in roots alone in darkness, 1.16 percent.

When potatoes were grown in the light the total quantity of solanine produced in tubers per hill was much greater than that produced in darkness, but there was very little difference in the percentage of solanine in the two lots of tubers. There is, however, a marked difference in the distribution of solanine under the two conditions.

In the light there is apparently a tendency for the alkaloid to concentrate in the aerial portion of the plant, while in the dark, solanine is fairly evenly distributed through tops and roots, with a slight tendency to approach a higher level in the roots.

The Earlaine variety was used in a study of the relation of length of day to solanine synthesis. Two photoperiods were employed, a long day of 18 hours, and a short day of 10 hours. In the long day, supplementary illumination was furnished with 60-watt Mazada lamps kept at a distance of 1.5 to 2 feet above the vines. Both sets of plants were grown in the same greenhouse. The night temperature ranged between 52° and 65° F., but was usually below 60°. During the day the temperature sometimes reached 108°; in most cases, however, the maximum was about 90° or less. The short-day plants were covered at sundown and ventilated with a fan which forced air out of the dark chamber, maintaining a temperature within 1° of the external air temperature. The chamber was taken down at the proper time the following morning. The experiment was allowed to run for a period of approximately 92 days, from February 2, 1939, to May 5, 1939.

In the short day, tuberization set in very early, and stem elongation was retarded. The final yield of potatoes was, however, lower than in the long day. The average height of the stems in the 10-hour day was 32 cm. Flower buds developed, but abscised without opening.

In the long day, tuberization was delayed, and long stolons were produced which in some cases pushed to the surface of the soil, becoming aerial branches. Stem elongation proceeded at a rapid rate, as is indicated by the fact that the average stem length on May 5 was 83.7 cm. All plants produced flowers, and while most of them abscised eight out of 21 hills set fruit.

Ten long-day plants and 18 short-day plants were pooled for solanine assay. Shoot tips, leaves, stems, stolons, roots, and tubers were analyzed separately. The shoot tips included side branches growing out of leaf axils, and consisted of approximately 2-cm. lengths of the terminal portions of the stems and branches. Average dry-weight data on the two day-length periods are given in table 6.

TABLE 6.—*Effect of photoperiod on the synthesis and distribution of solanine in the earlaine variety of potato*

Part of plant	Long day (18 hours)			Short day (10 hours)		
	Total solanine per hill	Solanine (milligrams per 100 grams of dry weight)	Dry weight per hill	Total solanine per hill	Solanine (milligrams per 100 grams of dry weight)	Dry weight per hill
	Milligrams		*Grams*	*Milligrams*		*Grams*
Shoot tips	1.6	229	0.71	0.28	173	0.16
Leaves	31.0	233	13.3	11.1	215	5.15
Stems [1]	2.6	43.0	5.93	.94	71	1.32
Stolons	6.0	708	.83	1.33	1,490	.09
Tubers	2.9	9.02	32.8	6.60	22.7	29.0
Roots	2.0	272	.73	1.10	480	.22
Total	46.1		54.30	21.35		35.94

[1] Exclusive of shoot tips.

The tubers were assayed in the fresh state, but for purposes of comparison with other tissues, the results are reported on a dry-weight basis. All other tissues were dried at 85° to constant weight. In the case of long-day tubers of Earlaine, the dry weight was 17.7 percent of the fresh weight, while in the short day, the dry weight was 19.4 percent.

There is a marked difference in the distribution of the solanine within the plant in the two lengths of day (table 6). In the long photoperiod, the solanine concentration in the shoot tips and leaves is higher than that in the corresponding organs of the 10-hour day. In the subterranean portions of the plant, however, the reverse is true, the short day material having a much higher concentration of the glycoside in the stolons, tubers, and roots than occurred in the same organs in the long day. The higher concentration of solanine in the short-day stems and stolons may be indicative of a high rate of translocation of the glycoside from the aerial regions to the underground portions of the plant, particularly to the tubers. In any case, the accumulation in growing tubers is here associated with the short-day check on vegetative growth. In the 18-hour day, approximately 70 percent of the solanine was in the aerial portions of the plant as against 58 percent in the 10-hour day.

Table 6 shows that the average total dry weight per hill, long day, is 54.3 gm.; whereas the average dry weight per hill, short day, is 35.9 gm. The percentage of solanine on the dry-weight basis is, long day, 0.085, and short day, 0.059.

A larger total solanine content per plant is found in the long day, but this is to be expected because of the greater dry weight in the 18-hour day. The solanine concentration, when expressed as a percentage of all tissues, however, remains higher in the long day than in the short day, indicating that increased synthesis of the glycoside had occurred in the long day.

Flowers were not analyzed in this experiment, but had this been done the difference between the percentage solanine content of plants in the different day lengths would have been still further emphasized, since flowers run high in solanine and only abortive buds were produced in the short day.

SEASONAL VARIATION IN SOLANINE CONTENT

The changes in solanine content of three varieties of potatoes were followed throughout the season. One early variety (Irish Cobbler) and two late varieties (White Rural and Russet Burbank) were used. Six samplings were made, beginning June 26, and continuing at 2-week intervals thereafter until September 1. This covered a period of from 22 to 88 days after the sprouts had emerged from the soil. The plants were collected in the field between 9 and 10 a. m.

Tubers were assayed in the fresh state by the usual method. Shoot tips, leaves, stems, and roots were analyzed separately. The shoot tips were cut to a length of about 2 cm., and these included the shoots arising from the leaf axils. After being dried at 85° C., these tissues were assayed for solanine by the recommended method, as described above. The results are indicated graphically in figure 9.

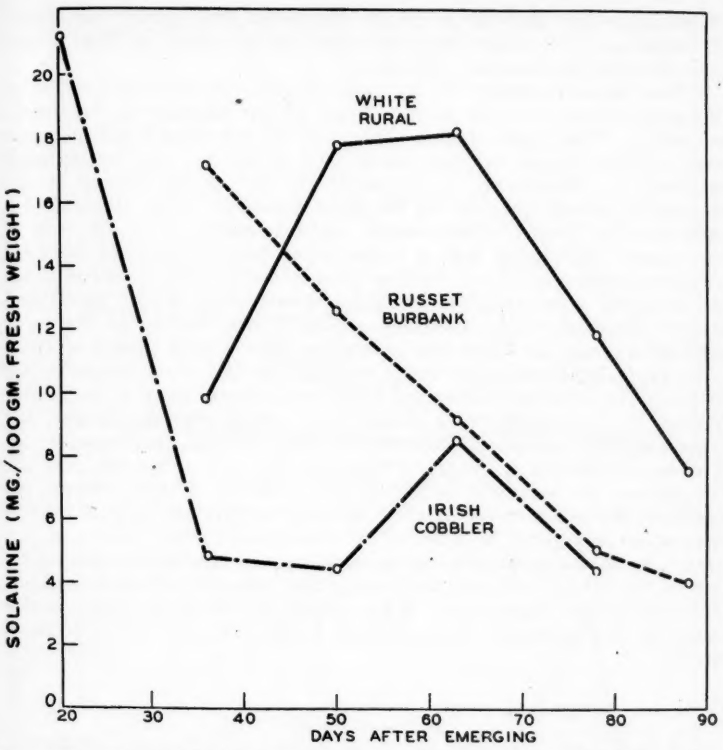

FIGURE 9.—Curves showing solanine content of tubers of three varieties of potatoes, at intervals during the growing season, fresh-weight basis.

In this figure, curves for the seasonal trends of the solanine concentration in the tubers of the three varieties are compared. These curves, it will be seen, differ radically in form. Irish Cobbler, an early variety, has a high initial solanine concentration when the average tuber weight is only 0.15 gm., and shows a slight secondary rise 63 days after emergence. However, the general level following the brief period of high solanine concentration at the beginning of the season is lower than in White Rural and Russet Burbank for the corresponding periods. Bömer and Mattis (*5*), who had sampled an early variety (Bohm's Allerfrüheste Gelbe) twice during the season, found that no change in solanine concentration occurred. They attributed this to the early ripening of the tubers, and believed that at the time of their first sampling (July 21) the tubers were almost ripe.

The White Rural, a late variety, showed a low initial solanine value. A maximum was reached after 50 days, and was maintained for about 2 weeks before dropping to lower levels with increasing maturity. Russet Burbank, another late variety, started out with a high level

of solanine but showed a steady decline as the season advanced. It cannot now be stated that such behavior is varietal, or that it may be influenced by seasonal differences.

These values represent the average solanine concentration of tubers of various sizes, since the average tuber weight increases as the season advances. The total solanine content of the tubers of a hill increases over a given period of time, as will be shown in text figures later referred to. Moreover, the forms of the curves are related to the respective growth behavior of the three varieties. The alkaloid concentration in Irish Cobbler, which matures early, levels off early in the season, indicating that a balance has been established between solanine accumulation and further tuber growth. On the other hand, the solanine concentration in the slow-maturing White Rural and Russet Burbank shows a steady decline corresponding to the more gradual increase in tuber size extending over a long period of time.

In table 7, the average tuber weights for the three varieties are listed. The average values are very low because only a small proportion of the tubers which are initiated reach marketable size, the large majority remaining between 2.5 and 7.5 mm. in diameter (*9*). A close correlation is observed between the rate of growth and the fluctuation in solanine concentration. White Rural tubers, for example, did not increase at all in average weight between 36 and 50 days after emergence because of the formation of new tubers at this time. In the same period (fig. 9), a sharp rise in solanine concentration of the tubers occurred, indicating that solanine synthesis was not retarded at the same time. When rapid growth in size was finally resumed, the solanine concentration leveled off and then declined rapidly.

TABLE 7.—*Average fresh weight of 3 varieties of potatoes per tuber at different periods of growth*

Variety	Days after emergence of shoots					
	22	36	50	63	78	88
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
White Rural	.....	6.32	5.0	9.66	20.2	31.6
Irish Cobbler	0.15	5.36	13.4	20.2	24.0	.....
Russet Burbank	.....	2.85	5.12	8.83	24.7	42.2

The solanine concentration of shoot tips, leaves, stems, roots, and tubers is compared on a dry-weight basis in figures 10, 11, and 12. In all three varieties, the shoot tips maintained the highest concentration throughout the season. Irish Cobbler and Russet Burbank showed a more or less continuous solanine decrease with time. In White Rural, however, there was an initial drop, coinciding with the period of most rapid growth, followed after 50 days by a rise in concentration. The maximum solanine concentration in this variety (2.37 gm. per 100 gm. of dry weight) was reached after 78 days, whereas both of the other varieties showed a peak value early in the season, about flowering time.

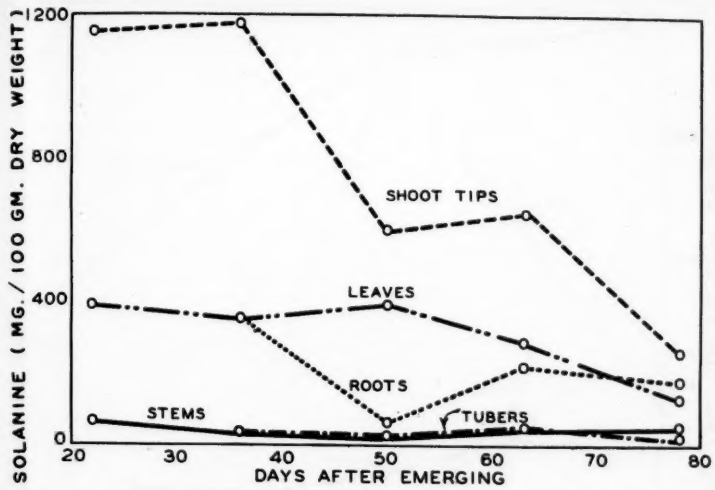

FIGURE 10.—Solanine content, dry-weight basis, of various parts of the developing potato plant, variety Irish Cobbler.

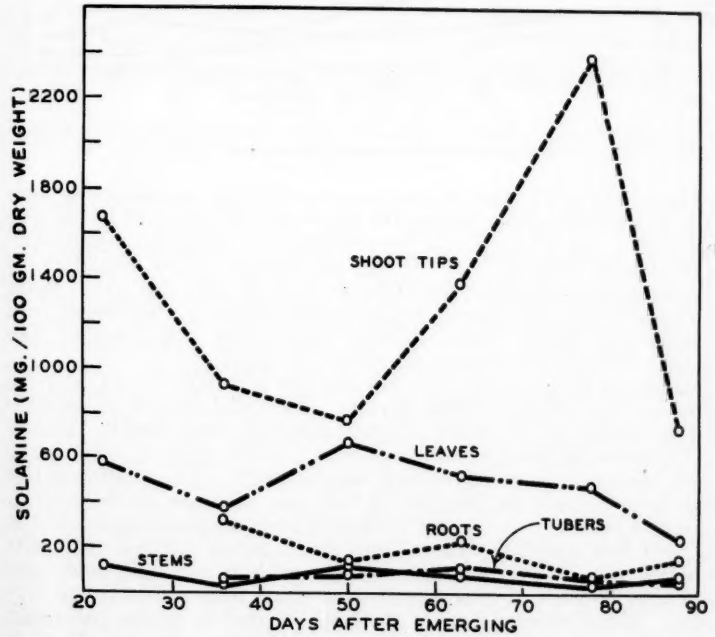

FIGURE 11.—Solanine content, dry-weight basis, of various parts of the developing potato plant, variety White Rural.

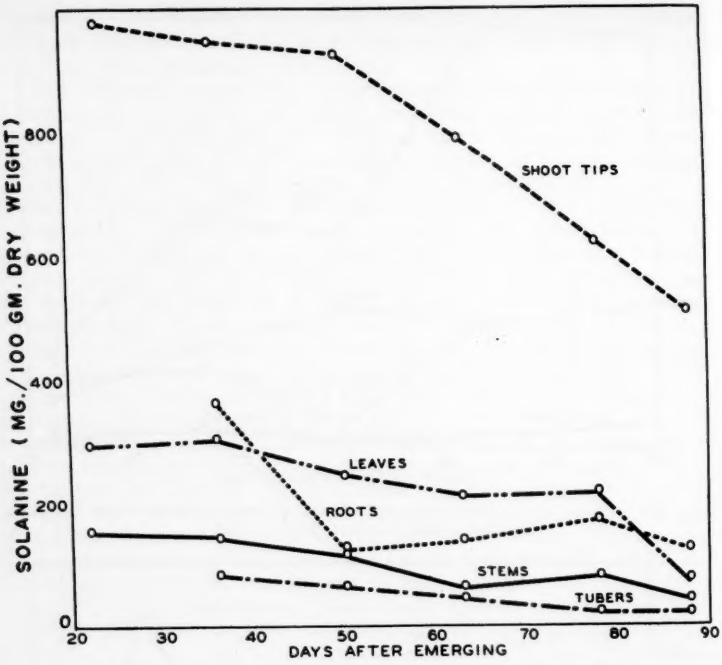

FIGURE 12.—Solanine content, dry-weight basis, of various parts of the developing potato plant, variety Russet Burbank.

The leaves in all cases showed an over-all drop in solanine content with minor fluctuations during the season to less than half of the original value. In White Rural, for example, the solanine concentration in the leaves dropped from 584 mg. per 100 gm. of dry weight at the first sampling to 230 mg. per 100 gm. after 88 days (fig. 11). Stems and tubers have the lowest concentration, which in the case of Irish Cobbler and White Rural parallel each other very closely. The changes in these two tissues are relatively small.

Measured in terms of total solanine per hill (figs. 13, 14, and 15) all three varieties show similar trends. The total amount of the alkaloid in the leaves and shoot tips increases with time; that is, the content increases as these organs increase in volume, reaching a maximum value, and then drops off more or less abruptly. The position of this peak with respect to time varies with the earliness of the variety. In Irish Cobbler, it is reached within 50 days after emergence, but in the very late maturing Russet Burbank it is not reached until after 78 days. In this variety, the solanine cycle is not fully completed even after 88 days, the full period over which these observations were made. Many observations of the type referred to in this paragraph make it appear probable that, in general, higher solanine content is associated with higher metabolic activity.

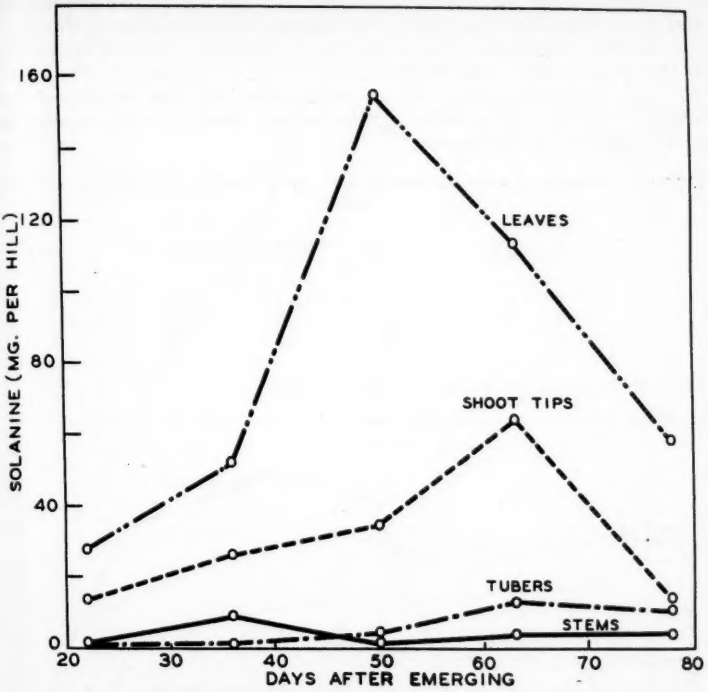

FIGURE 13.—Solanine content of various parts of the developing potato plant, variety Irish Cobbler.

Some idea of the manner in which the observed solanine changes occur in leaves is gained from table 8. The leaves used in this study were taken from greenhouse-grown Earlaine plants at three different positions on the stem. The first sample included the oldest leaves, and was taken from the first and second nodes at the base of the stem. The second and third samples were made up of leaves taken from the seventh and eighth nodes and the thirteenth to fifteenth nodes, respectively. As the total dry weight per leaf increases in passing from nodes 13 to 15 down to nodes 7 or 8, the total solanine content per leaf keeps pace with the increase in weight, reaching a maximum of 0.913 mg. per leaf. It will be noted that the maximum dry weight per leaf is also reached at the seventh and eighth nodes. On a percentage basis, the solanine content remains almost unchanged at the two levels. Solanine apparently continues to be produced by the leaf until full size has been reached. Further increase in age of the leaf results in a loss of dry weight which is accompanied by a sharp drop in total as well as in the percentage solanine in the leaf. The observed loss in the amount of the glycoside present in the leaves may be brought about by translocation, or the substance may be metabolized. This disappearance of solanine from the lower, older leaves

explains in large part the abrupt drop in both solanine concentration and total solanine observed in the leaves of field plants at the end of the season. Also, since leaves of various ages were mixed for analysis in the experiments summarized graphically in figures 13, 14, and 15, the loss of solanine in the old leaves explains why the maximum total solanine content of leaves is reached much earlier in the season than in the case of the shoot tips.

TABLE 8.—*Change of solanine content with age of leaf in the Earlaine variety of potato*

Node No.[1]	Dry weight per leaf	Solanine per leaf	Solanine, dry weight
	Grams	*Milligrams*	*Percent*
13–15	0.182	0.278	0.152
7–8	.542	.913	.168
1–2	.502	.502	.100

[1] Node No. 1 is taken as the first node at the base of the stem.

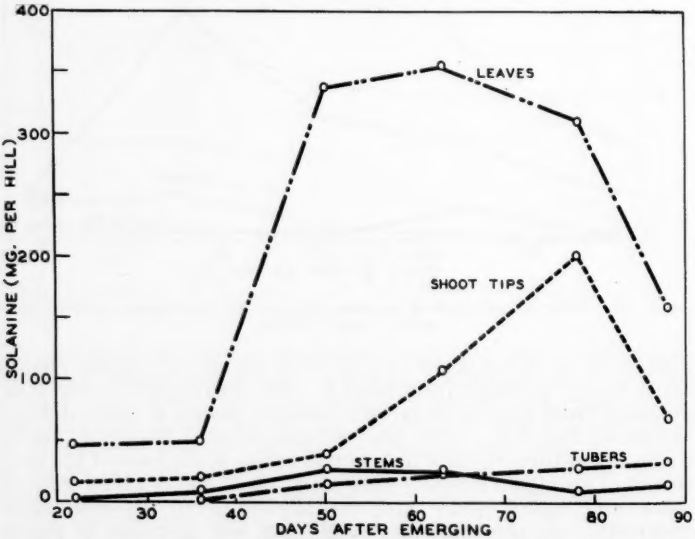

FIGURE 14.—Solanine content of various parts of the developing potato plant, variety White Rural.

The solanine content of stems fluctuated somewhat, but generally remained at a comparatively low level throughout the season (figs. 13 to 15). The tubers were the only structures which showed a steady accumulation of total solanine per tuber over the entire period, suggesting that translocation was occurring from the aerial portions of the plant. Even assuming that all of the solanine found in the tubers in the last sampling was supplied by transport from the tops, only a small fraction of the solanine which disappeared from the aerial organs can be so accounted for in White Rural and Irish Cobbler (table 9).

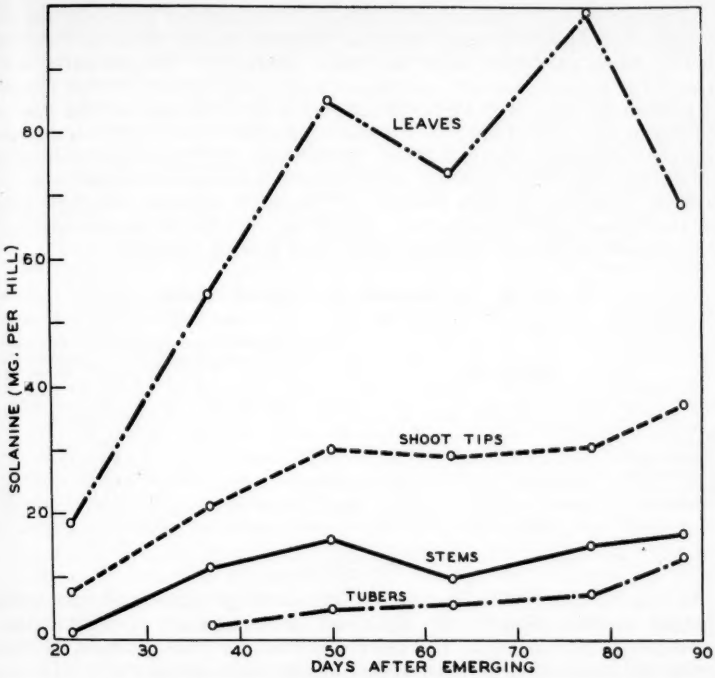

FIGURE 15.—Solanine content of various parts of the developing potato plant, variety Russet Burbank.

In Russet Burbank, slightly more than one-half the amount of solanine lost from the tops can be accounted for on the assumption that it is translocated to the tubers. Under Wisconsin conditions, however, Russet Burbank matures so very slowly that at the last sampling on September 1 the solanine content of the shoot tips was still rising, and that of the leaves had just begun to decline, so that no final conclusions can be drawn respecting this variety.

TABLE 9.—*Solanine changes in tops and tubers*

Variety	Maximum solanine content of tops	Final solanine content of tops	Difference between maximum and final solanine levels	Maximum solanine content of tubers
	Milligrams per hill	*Milligrams per hill*	*Milligrams per hill*	*Milligrams per hill*
Russet Burbank	144	122	22.0	12.9
White Rural	534	250	284	36.3
Irish Cobbler	172	77.6	95.3	10.9

The leaves of *S. commersonii*, which were sampled twice during the season, had about the same solanine content on July 29 as on September 27 when the plants were harvested (table 10). As compared with the three potato varieties discussed above, all organs of this species maintain an extremely high solanine level up to the end of the season. Stems in the usual varieties of potato normally have a low concentration of the alkaloid (26.5–108 mg. per 100 gm. of dry weight in the case of the variety White Rural), whereas in *S. commersonii* stems contain almost 1 percent of this product. This high solanine concentration in the stems may be indicative merely of the rate of translocation to the roots and tubers, which also run very high in solanine.

TABLE 10.—*Solanine content of Solanum commersonii*

Part of plant	Solanine (grams per 100 gm. of dry weight)—		Solanine (grams per 100 gm. of fresh weight)
	July 29, 1940	Sept. 27, 1940	
Leaves	2.610	2.635	
Upper stems		.962	
Aerial stolons		2.467	
Roots		1.230	
Tubers		1.445	0.452

DISCUSSION

So far as space would permit, the chemical phases of the work treated in this paper were discussed in connection with the data presented. Accordingly, the brief observations incorporated at this point will be limited to a few physiological considerations.

It would seem to be apparent that the relative decrease in solanine concentration which is normally observed in maturing tubers is to a large extent the result of the diluting effect of the growth of the tubers, since there is an increase in total solanine over the same period. Environmental factors must also be considered, and the growth element is probably dominant. Not infrequently there is, following a period of water deficiency, a marked renewal of growth activity in the tuber during the late season. The effect of these changes on solanine content is not known. Maturation and senescence in aerial organs bring about a decrease in solanine, apparently as a result of metabolic utilization, and no doubt, at least to some extent, through transport to subterranean organs.

Data for total solanine content of roots are not available, since an estimation of the total root systems of field plants requires special facilities. It is unlikely, however, that very much of the solanine lost from the tops can be accounted for on the assumption that it is transported to the roots. The root system is almost fully developed by the time the decline in total solanine becomes apparent, and, furthermore, the percentage solanine concentration in roots and stems during the period when such transport would be occurring shows no significant upward trend. In view of these facts, it appears best to assume that the glycoside is to some extent metabolized toward the end of the growing season. This view is substantiated by the obser-

vation that at the end of the growing season the brown, wilted potato leaves contain solanidine, indicating conversion of a part of the glycoside to the aglycone (*16*).

The percentage solanine concentration of the varieties listed in table 4 should not be regarded as absolute. From the data thus far available, it would seem that the concentration of the glycoside displays considerable lability in response to environmental conditions such as soil types, temperature, and mineral nutrition, but the values reported do nevertheless fall within the normal range of concentration which might be expected under standard field conditions.

The unusually high solanine concentrations of otherwise normal potato tubers reported by some workers (*5*, *10*, *21*, *23*, *24*) has never been satisfactorily explained. The marked decrease of solanine in old leaves and the depletion trend in the entire aerial portion of the plant toward the end of the growing season, without its reappearance in the roots or tubers in equivalent amount, further indicate that solanine is destructively metabolized during the development of the plant. It might also be pointed out that if the quantities of the glycoside which disappeared from the tops (table 9) had been translocated to the tubers, their solanine concentration would easily have reached the toxic levels sometimes reported in potatoes, as referred to above. In the variety White Rural, for example, the 284 mg. of solanine which disappeared from the aerial organs would have resulted in a solanine concentration of 71 mg. per 100 gm. of fresh tuber weight, assuming a yield of 400 gm. of potatoes per hill. It is conceivable that environmental conditions may at times greatly modify the export of solanine to the tubers. Moreover, the glycoside is sufficiently soluble at the pH of potato sap to justify the assumption that it may be readily transported in the plant.

In view of the high solanine content of the leaves of cultivated varieties of potatoes, it is, in general, reassuring to find that there is some natural block to the transport of the alkaloid to tubers, but until more is known about the effectiveness of this block it appears wise, especially with new varieties, to keep watch of this potential danger of tuber accumulation. Meanwhile, the problem may be experimentally attacked.

Since small-tuber analyses of *Solanum commersonii* indicate a solanine concentration 39 times that of tubers of White Rural of average size, tubers of the former species must be classed as definitely toxic; also in the raw state they are bitter and irritating to the taste. The species should be looked upon with suspicion as a basis of new stock in potato breeding whether for quality or disease resistance.

SUMMARY

The extraction of the potato alkaloids from leaves and sprouts and their separation and purification are described. Some properties of the potato alkaloids are discussed. Solanine is insoluble in water above pH 9.3, making it unnecessary to apply a correction for solubility in the quantitative estimation of the alkaloid if the pH during precipitation is maintained above pH 9.3.

A modified Bömer and Mattis procedure for the quantitative determination of solanine in fresh potato tubers is described. The

colorimetric procedure developed by Alberti and by Pfankuch for potato tubers was adapted to the quantitative estimation of solanine in aqueous extracts of leaves, stems, roots, and shoot tips.

In normal tubers, solanine is found in the highest concentration in the peripheral zone comprising the periderm and cortical parenchyma. Little or none is found in the pith, and only small amounts occur in the intermediate region. From a survey of 32 varieties of potatoes grown under Wisconsin conditions during one season, the solanine concentration was found to range from about 2 to 13 mg. per 100 gm. of fresh tuber, while the tubers of *Solanum commersonii* had as much as 500 mg per 100 gm. of tissue.

From tests with certain standard potato varieties it was found that in the new plants produced from the tubers solanine can be synthesized in the dark, and only a slightly greater solanine production is observed in the light. In the dark, solanine is fairly evenly distributed between aerial and subterranean parts of the plant, while in the light it tends to concentrate in the tops. The photoperiod affects both the synthesis and the distribution of solanine. In a 10-hour day, the solanine content was 0.059 percent of the total dry weight as against 0.085 percent in a long day of 18 hours. In the short day approximately 58 percent of the glycoside was found in the aerial portion of the plant as against 70 percent in the long day.

The percentage concentration of solanine in potato tubers declines as the season advances. In the early variety, Irish Cobbler, there is an abrupt drop early in the season, after which a low level is maintained for the remainder of the growth period. In the late variety, White Rural, there is an initial rise to a maximum concentration which is maintained for about 2 weeks, and this is followed by a drop. The slowly maturing Russet Burbank shows a steady decline in concentration throughout the season. In all three varieties, the total solanine per hill accumulates with time. Throughout the season, the highest solanine concentration is maintained by the shoot tips. The leaves, exclusive of the very young ones at the stem tips, are intermediate in concentration, and in all cases show an over-all drop in concentration of the alkaloid. The concentration in the older roots is in most cases lower than that in the leaves. Expressed on a dry-weight basis, the solanine content of tubers and of aerial stems is approximately of the same order, and in both organs it remains relatively low throughout the season in all three varieties.

In all varieties, by far the greater part of the solanine per hill is contained in the leaves. The total solanine curves for both leaves and shoot tips show well-defined maxima. The total solanine in aerial stems and in tubers is very low compared with that found in other organs of the plant. Tubers are the only organs which show a continuous accumulation of solanine throughout the season, exhibiting a slight but steady rise with time. Aerial stems display only minor fluctuations in alkaloid concentration. In all of the varieties tested, there was a much greater loss of solanine from the tops than can be accounted for on the basis of transport to the underground portions of the plant. In White Rural, approximately 284 mg. disappeared from the tops, whereas the maximum solanine content of tubers was

only about 36 mg. The glycoside is apparently metabolized toward the end of the growing season.

The leaves of *Solanum commersonii* have a solanine concentration of about 2.6 percent on a dry-weight basis, and show no appreciable variation in concentration between July and September.

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U. S. GOVERNMENT PRINTING OFFICE: 1946

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